Gel Purification

Aim

To purify DNA from gel, usually to obtain either DNA fragments of a certain length or to eliminate contaminating proteins and salts.

Procedure

Before using this protocol add ethanol (96-100 %) to Buffer PE (See instructions on the bottle). Add 1:250 volume pH indicator I to buffer PB. The yellow color of Buffer PB with pH indicator I indicates a pH $\leq$ 7.5. The adsorption of DNA to the membrane is only efficient at $\leq$ 7.5.

1. Cut out the DNA fragment from the agarose gel with a scalpel.
2. Weigh the the gel slice in a colorless tube. Add 3 volumes Buffer QG to 1 volume gel (100 mg $\sim$ 100 μl gel). The maximum amount of gel per spin column is 400 mg. For $>$ 2 % use 6 volumes Buffer QG.
3. Incubate at 50 °C for 10 min (or until the gel slice has completely dissolved).
   Vortex the tube every 2-3 min to help dissolve gel. After the gel slice has dissolved completely, check that the color of the mixture is yellow (similar to Buffer QG without dissolved agarose). If the color is orange or violet, add 10 μl 3 M sodium acetate, pH 5.0, and mix. The mixture turns yellow.
4. Add 1 volume isopropanol to the sample and mix.
5. Place a spin column in a provided collection tube. To bind DNA, apply the sample to the spin column and centrifuge for 1 min, 13 000 rpm. Discard flow-through and place the spin column back into the collection tube.
6. If the DNA will subsequently be used for sequencing, in vitro transcription, or microinjection, add 500 μl Buffer QG to the spin column and centrifuge for 1 min. Discard flow-through and place the spin column back into the same tube.
7. To wash, add 750 μl Buffer PE to the spin column and centrifuge for 1 min. Discard flow-through and place the spin column into the same tube.
   Note: If the DNA will be used for salt-sensitive applications (e.g., sequencing, blunt-ended ligation), let the column stand for 2-5 min after addition of Buffer PE.

   Centrifuge the spin column in the collection tube for 1 min, 13 000 rpm to remove residual wash buffer.
8. Place the spin column in a clean Eppendorf tube.
9. To elute DNA, add 30 μl Buffer EB (10 mM Tris-HCl, pH 8.5) or water to the center of the membrane. Let the column stand for 4 min and centrifuge for 1 min, 13 000 rpm.

Notes!

This protocol is used for purification of up to 10 μg of DNA (100 bp to 10 kbp).

Sources

This protocol is originally distributed from QIAGEN and can be found at https://www.qiagen.com/ie/resources/resourcedetail?id=3987caa6-ef28-4abd-927e-d5759d986658&lang=en (retrieved 04.10.2016) Modifications have been made to the original protocol to adapt it to our applications.